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S P E C I F I C A T I O N

COMPOUNDS HAVING SELECTIVE ACTIVITY FOR RETINOID X RECEPTORS, AND MEANS FOR MODULATION OF PROCESSES MEDIATED BY RETINOID X RECEPTORS

RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 944,783, filed on September 11, 1992, which is a continuation-in-part of application Serial No. 872,707 filed April 22, 1992, whose entire disclosures are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to intracellular receptors and ligands therefor. More specifically, this invention relates to compounds having selective activity for specific retinoic acid receptors, and methods for use of such compounds.

BACKGROUND OF THE INVENTION

The vitamin A metabolite retinoic acid has long been recognized to induce a broad spectrum of biological effects. A variety of structural analogues of retinoic acid have been synthesized that also have been found to be bioactive. Some, such as Retin-A® (registered trademark of Johnson & Johnson) and Accutane® (registered trademark of Hoffmann-LaRoche), have found utility as therapeutic agents for the treatment of various pathological conditions. Metabolites of vitamin A and their synthetic analogues are collectively herein called "retinoids".

Synthetic retinoids have been found to mimic many of the pharmacological actions of retinoic acid. However, the broad spectrum of pharmacological actions of retinoic acid is not reproduced in full by all bioactive synthetic retinoids.

Medical professionals have become very interested in the medicinal applications of retinoids. Among their uses approved by the FDA is the treatment of severe forms of acne and psoriasis. A large body of evidence also exists that these compounds can be used to arrest and, to an extent, reverse the effects of skin damage arising from prolonged exposure to the sun. Other evidence exists that these compounds may be useful in the treatments of a variety of severe cancers including melanoma, cervical cancer, some forms of leukemia, and basal and squamous cell carcinomas. Retinoids have also shown an ability to be efficacious in treating premalignant cell lesions, such as oral leukoplakia, and to prevent the occurrence of malignancy.

Use of the retinoids is associated with a number of significant side effects. The most serious of these is that, as a class, they are among the most potent teratogens known. Teratogens are compounds that cause severe birth defects during specific periods of fetal exposure. Other side effects include irritation of the tissues treated, which can be so severe that patients cannot tolerate treatment.

Various investigations have been undertaken to elucidate the structure-activity relationships governing the abilities of synthetic retinoids to induce the various pharmacological consequences of retinoic acid exposure. This has been a complicated task, however, since the assays available to investigators have been bioassays, carried out either in intact animals or in isolated tissues. Technical constraints have often dictated the use of different small animal species for different

assays. Interpretation of results has been complicated by possible pharmacokinetic and metabolic effects and possible species differences in the receptors involved. Nevertheless, definite differences in the pharmacological effects of various synthetic retinoids have been observed.

Major insight into the molecular mechanism of retinoic acid signal transduction was gained in 1988. Prior to that time, several high abundance cellular retinoid binding proteins were incorrectly inferred to be the signal transducing receptors for retinoic acid. In 1988, a member of the steroid/thyroid hormone intracellular receptor superfamily (Evans, Science, 240:889-95 (1988)) was shown to transduce a retinoic acid signal (Giguere et al., Nature, 330:624-29 (1987); Petkovich et al., Nature, 330: 444-50 (1987)). This unexpected finding related retinoic acid to other non-peptide hormones and elucidated the mechanism of retinoic acid effects in altering cell function. It is now known that retinoids regulate the activity of two distinct intracellular receptor subfamilies; the Retinoic Acid Receptors (RARs) and the Retinoid X Receptors (RXRs).

The first retinoic acid receptor identified, designated RAR-alpha, acts to modulate transcription of specific target genes in a manner which is ligand-dependent, as has been shown to be the case for many of the members of the steroid/thyroid hormone intracellular receptor superfamily. The endogenous low-molecular-weight ligand upon which the transcription-modulating activity of RAR-alpha depends is all-<u>trans</u>-retinoic acid. Retinoic acid receptor-mediated changes in gene expression result in characteristic alterations in cellular phenotype, with consequences in many tissues manifesting the biological response to retinoic acid. Two additional genes closely related to RAR-alpha were recently identified and were designated RAR-beta and

RAR-gamma and are very highly related (Brand et al., Nature, 332:850-53 (1988); Ishikawa et al., Mol. Endocrin., 4:837-44 (1990)). In the region of the retinoid receptors which can be shown to confer ligand binding, the primary amino acid sequences diverge by less than 15% among the three RAR subtypes or isoforms. All-trans-retinoic acid is a natural ligand for the retinoic acid receptors (RARs) and is capable of binding to these receptors with high affinity, resulting in the regulation of gene expression. The newly-discovered retinoid metabolite, 9-cis-retinoic acid, is also an activator of RARs.

A related but unexpected observation was made recently (Mangelsdorf et al., Nature, 345:224-29 (1990)), in which another member of the steroid/thyroid receptor superfamily was also shown to be responsive to retinoic acid. This new retinoid receptor subtype has been designated Retinoid X Receptor (RXR), because certain earlier data suggested that a derivative of all-trans-retinoic acid may be the endogenous ligand for RXR. Like the RARs, the RXRs are also known to have at least three subtypes or isoforms, namely RXR-alpha, RXR-beta, and RXR-gamma, with corresponding unique patterns of expression (Manglesdorf et al., Genes & Devel., 6:329-44 (1992)).

Although both the RARs and RXRs respond to all-trans-retinoic acid in vivo, the receptors differ in several important aspects. First, the RARs and RXRs are significantly divergent in primary structure (e.g., the ligand binding domains of RARα and RXRα have only 27% amino acid identity). These structural differences are reflected in the different relative degrees of responsiveness of RARs and RXRs to various vitamin A metabolites and synthetic retinoids. In addition, distinctly different patterns of tissue distribution are seen for RARs and RXRs. For example, in contrast to the RARs, which are not expressed at high

levels in the visceral tissues, RXRα mRNA has been shown to be most abundant in the liver, kidney, lung, muscle and intestine. Finally, the RARs and RXRs have different target gene specificity. For example, response elements have recently been identified in the cellular retinal binding protein type II (CRBPII) and apolipoprotein AI genes which confer responsiveness to RXR, but not RAR. Furthermore, RAR has also been recently shown to repress RXR-mediated activation through the CRBPII RXR response element (Manglesdorf et al., Cell, 66:555-61 (1991)). These data indicate that two retinoic acid responsive pathways are not simply redundant, but instead manifest a complex interplay. Recently, Heyman et al. (Cell, 68:397-406 (1992)) and Levin et al. (Nature, 355:359-61 (1992)) independently demonstrated that 9-cis-retinoic acid is a natural endogenous ligand for the RXRs. 9-cis-retinoic acid was shown to bind and transactivate the RXRs, as well as the RARs, and therefore appears to act as a "bifunctional" ligand.

In view of the related, but clearly distinct, nature of these receptors, ligands which are more selective for the Retinoid X Receptor subfamily would be of great value for selectively controlling processes mediated by one or more of the RXR isoforms, and would provide the capacity for independent control of the physiologic processes mediated by the RXRs. Ligands which preferentially affect one or more but not all of the receptor isoforms also offer the possibility of increased therapeutic efficacy when used for medicinal applications.

The entire disclosures of the publications and references referred to above and hereafter in this specification are incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention is directed to compounds, compositions, and methods for modulating processes mediated by one or more Retinoid X Receptors. More particularly, the invention relates to compounds which selectively or preferentially activate Retinoid X Receptors, in comparison to Retinoic Acid Receptors. These compounds selectively modulate processes mediated by Retinoid X Receptors. Accordingly, the invention also relates to methods for modulating processes selectively mediated by one or more Retinoid X Receptors, in comparison to Retinoic Acid Receptors, by use of the compounds of this invention. Examples of compounds used in and forming part of the invention include bicyclic benzyl, thiophene, furanyl, and pyrrole derivatives. Pharmaceutical compositions containing the compounds disclosed are also within the scope of this invention. Also included are methods for identifying or purifying Retinoid X Receptors by use of the compounds of this invention.

BRIEF DESCRIPTION OF THE FIGURES

The present invention may be better understood and its advantages appreciated by those skilled in the art by referring to the accompanying drawings wherein:

Figure 1 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNCB.

Figure 2 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by all-trans-retinoic acid.

Figure 3 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 9-cis-retinoic acid.

Figure 4 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNEB.

Figure 5 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-bromo-TTNEB.

Figure 6 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNCHBP.

Figure 7 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNEHBP.

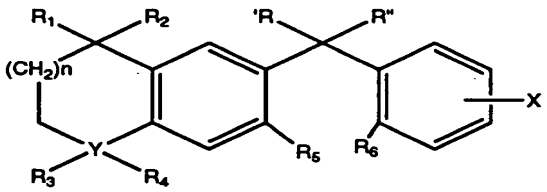
Figure 8 presents the inhibition of transglutaminase activity by 9-cis-retinoic acid, all-trans-retinoic acid, and 3-methyl-TTNCB.

Figure 9 presents the Topical Dose Response, based on the test on Rhino mice, for 9-cis-retinoic acid, all-trans-retinoic acid, and 3-methyl-TTNCB.

Figure 10 presents the effect on rat HDL cholesterol of all-trans-retinoic acid, 9-cis-retinoic acid, 3-methyl-TTNCB, and 3-methyl-TTNEB.

DETAILED DESCRIPTION OF THE INVENTION

This invention discloses retinoid-like compounds or ligands which have selective activity for members of the subfamily of Retinoid X Receptors (RXRs), in comparison to members of the subfamily of Retinoic Acid Receptors (RARs). Examples of such compounds are bicyclic benzyl, thiophene, furanyl, and pyrrole derivatives which can be represented by the formulae:



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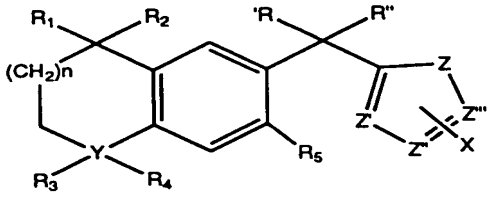

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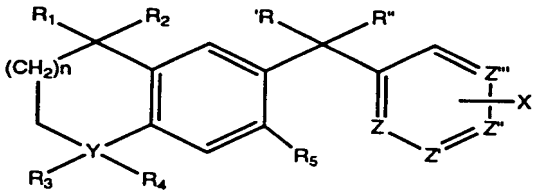

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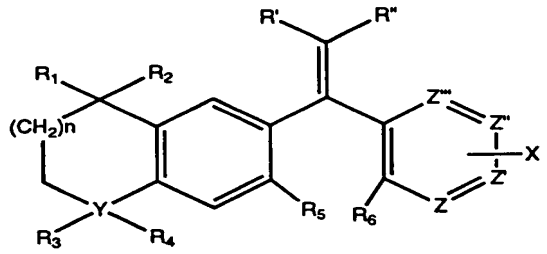

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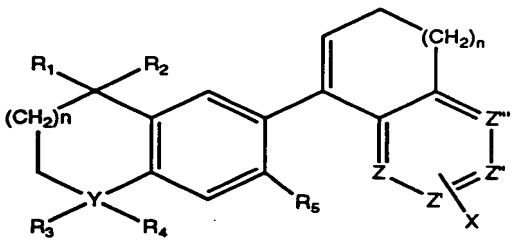
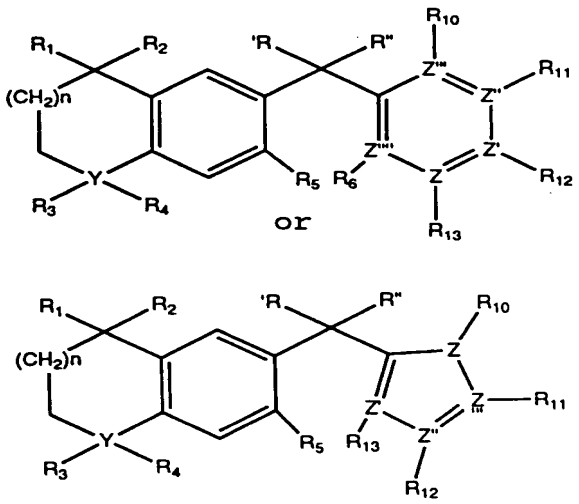




wherein

R_1 and R_2, each independently, represent hydrogen or lower alkyl or acyl having 1-4 carbon atoms;

Y represents C, O, S, N, or a pharmaceutically acceptable salt;

R_3 represents hydrogen or lower alkyl having 1-4 carbon atoms where Y is C or N, but R_3 does not exist if Y is O or S;

R_4 represents hydrogen or lower alkyl having 1-4 carbon atoms where Y is C, but R_4 does not exist if Y is O, N, or S;

R′ and R" represent hydrogen, lower alkyl or acyl having 1-4 carbon atoms, OH, alkoxy having 1-4 carbon atoms, thiol or thio ether, or amino,

or R′ or R" taken together form an oxo, methano, thioketone, hydroxy amino, epoxide, or cyclopropyl group;

R_5 represents hydrogen, a lower alkyl having 1-4 carbons, halogen, nitro, OR_7, SR_7, NR_7R_8, or $(CF)_nCF_3$;

R_6, R_{10}, R_{11}, R_{12}, R_{13} each independently represent hydrogen, a lower alkyl having 1-4 carbons, halogen, nitro, OR_7, SR_7, NR_7R_8 or $(CF)_nCF_3$, and exist only if the Z, Z′, Z", Z′", or Z"" from which it originates is C, or each independently represent hydrogen or a lower alkyl having 1-4 carbons if the Z, Z′, Z", Z′", or Z"" from

which it originates is N, and R_6 and R_{10} cannot both be H if R_5 is H, and where one of R_6, R_{10}, R_{11}, R_{12} or R_{13} is X;

R_7 represents hydrogen or a lower alkyl having 1-6 carbons;

R_8 represents hydrogen or a lower alkyl having 1-6 carbons;

X is COOH, tetrazole, PO_3H, SO_3H, CHO, CH_2OH, $CONH_2$, COSH, $COOR_9$, $COSR_9$, $CONHR_9$, or COOW where R_9 represents a lower alkyl having 1-4 carbons, phenyl, or m-hydroxyphenyl, m-bromophenyl, m-chlorophenyl, m-florophenyl, or m-iodophenyl, where m=2-4, where W is a pharmaceutically acceptable salt, and where X can originate from any C or N on the ring;

Z, Z', Z", Z"' and Z"", each independently, represent C, S, O, N, or a pharmaceutically acceptable salt; and

n = 0-3.

As used in this disclosure, pharmaceutically acceptable salts include but are not limited to: hydrochloric, hydrobromic, hydroiodic, hydrofluoric, sulfuric, citric, maleic, acetic, lactic, nicotinic, succinic, oxalic, phosphoric, malonic, salicylic, phenylacetic, stearic, pyridine, ammonium, piperazine, diethylamine, nicotinamide, formic, urea, sodium, potassium, calcium, magnesium, zinc, lithium, cinnamic, methylamino, methanesulfonic, picric, tartaric, triethylamino, dimethylamino, and tris(hydroxymethyl)aminomethane. Additional pharmaceutically acceptable salts are known to those of skill in the art.

Representative derivatives according to the present invention include the following:

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-carbonyl)]-benzoic acid, designated "3-methyl-TTNCB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-isopropyl-2-naphthyl-(2-carbonyl)]-benzoic acid, designated "3-IPR-TTNCB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-isopropyl-2-naphthyl-(2-methano)]-benzoic acid, designated "3-IPR-TTNEB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-ethyl-2-naphthyl-(2-methano)]-benzoic acid, designated "3-ethyl-TTNEB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-bromo-2-naphthyl-(2-methano)]-benzoic acid, designated "3-bromo-TTNEB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-chloro-2-naphthyl-(2-methano)]-benzoic acid, designated "3-chloro-TTNEB";

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-methano)]-benzoic acid, designated "3-methyl-TTNEB";

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-hydroxy-methyl)]-benzoic acid, designated "3-methyl-TTNHMB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-bromo-2-naphthyl-(2-carbonyl)]-benzoic acid, designated "3-bromo-TTNCB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-chloro-2-naphthyl-(2-carbonyl)]-benzoic acid, designated "3-chloro-TTNCB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-hydroxy-2-naphthyl-(2-carbonyl)]-benzoic acid, designated "3-hydroxy-TTNCB";

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-ethyl-2-naphthyl-(2-carbonyl)]-benzoic acid, designated "3-ethyl-TTNCB";

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-thioketal)]-benzoic acid, designated "thioketal";

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-thioketo)]-benzoic acid, designated "thioketone";

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-carbonyl)]-N-(4-hydroxyphenyl)benzamide, designated "3-methyl-TTNCHBP";

p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-methano)]-N-(4-hydroxyphenyl)benzamide, designated "3-methyl-TTNEHBP";

2[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) ethenyl] pyridine-5-carboxylic acid, designated "TPNEP";

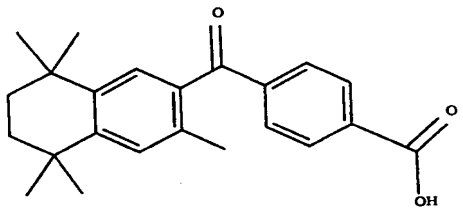
ethyl-2[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) ethenyl] pyridine-5-carboxylate, designated "TPNEPE";

2[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl) ethenyl] pyridine-5-carboxylic acid, designated "TTNEP";

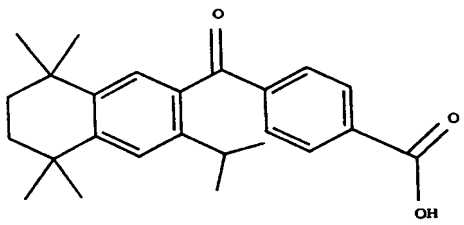
4[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) epoxy] benzoic acid, designated "TPNEB"; and

4[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) cyclopropyl] benzoic acid, designated "TPNCB".

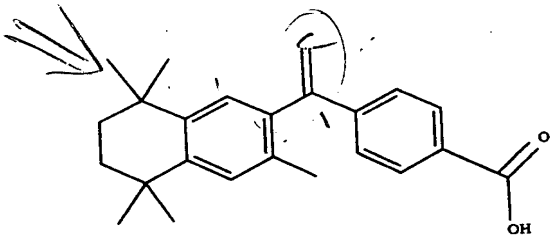
Examples of structures for such compounds are as follows:



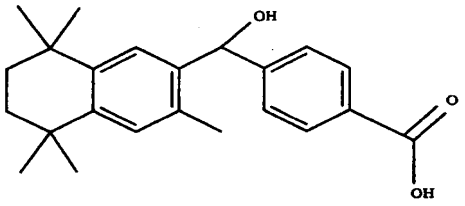
3-methyl-TTNCB



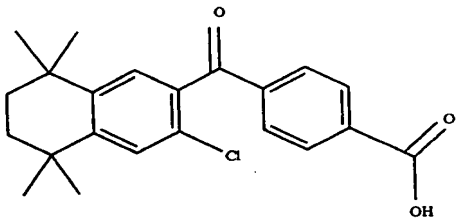
3-IPR-TTNCB



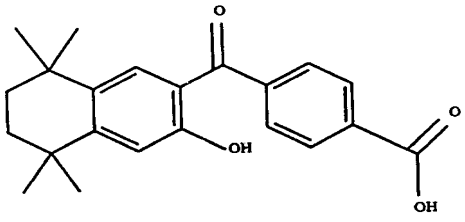
3-methyl-TTNEB



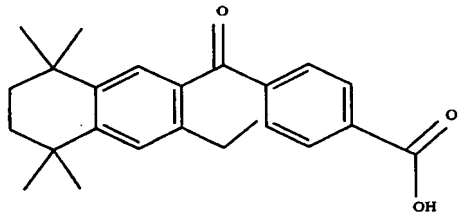
3-methyl TTNHMB



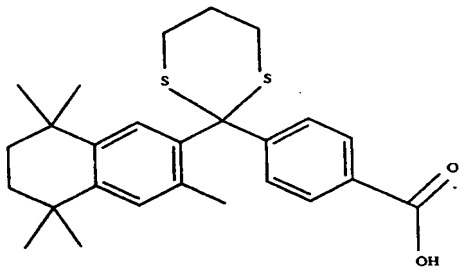
3-chloro-TTNCB



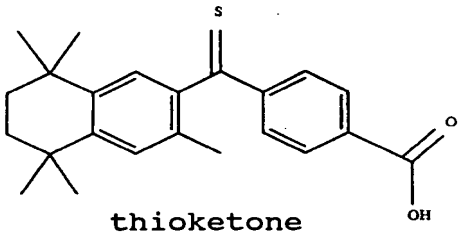
3-hydroxy-TTNCB



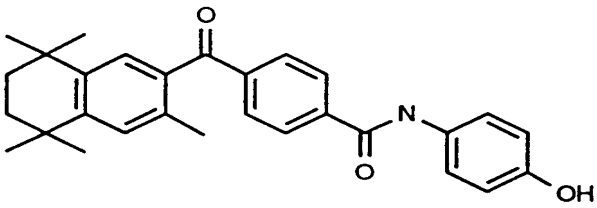
3-ethyl-TTNCB



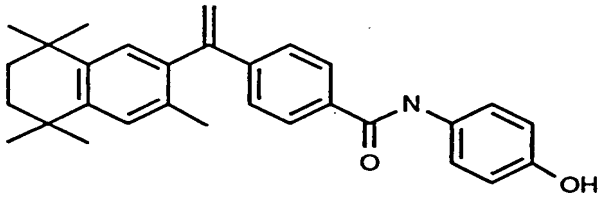
thioketal



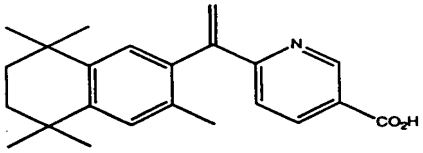
thioketone



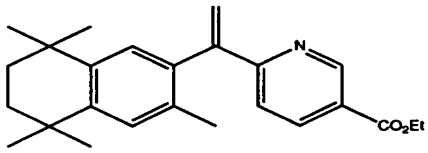
3-methyl-TTNCHBP



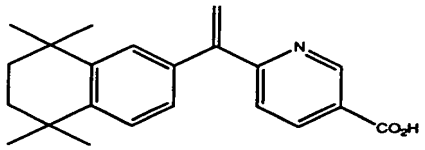
3-methyl-TTNEHBP



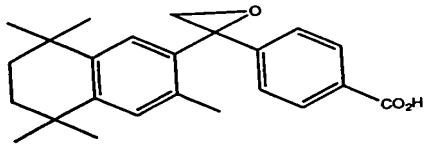
TPNEP



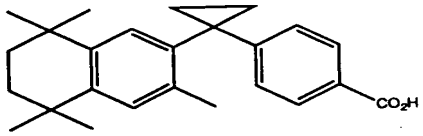
TPNEPE



TTNEP



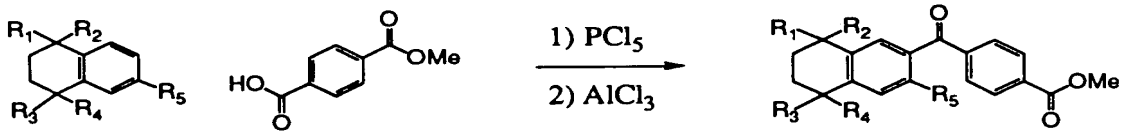
TPNEB



TPNCB

In addition, thiophene, furanyl, pyridine, pyrazine, pyrazole, pyridazine, and pyrrole groups function as isosteres for phenyl groups, and may be substituted for the phenyl group of the above bicyclic benzyl derivatives.

Representative derivatives of the present invention can be prepared according to the following illustrative synthetic schemes:



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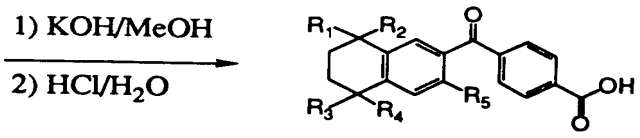
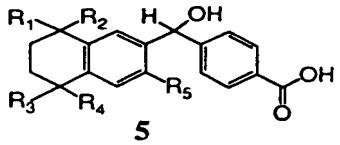


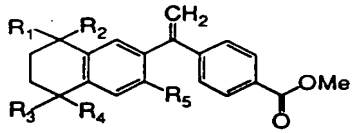
4

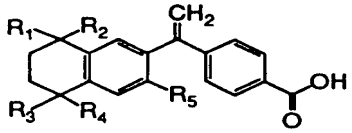
Compounds of structure **1** containing R_5 = lower alkyl are prepared in accordance with United States Patent No. 2,897,237. When R_5 = Halo, OH, amino, or thio, the products are prepared by standard Friedel-Crafts reaction conditions combining the appropriate substituted benzene with 2,5-dichloro-2,5-dimethyl hexane in the presence of aluminum trichloride.

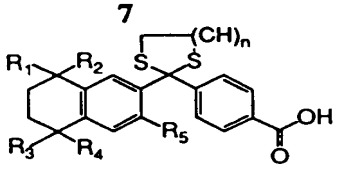
Condensation of **1** with mono-methyl terephthalate **2** was carried out by addition of PCl_5 to **1** and **2** in CH_2Cl_2 followed by addition of $AlCl_3$ at room temperature.

The resulting methyl esters **3** are hydrolyzed to the carboxylic acid **4** by refluxing in aqueous KOH-MeOH followed by acidification.

4 $\xrightarrow[2)\ HCl/H_2O]{1)\ NaBH_4/MeOH}$  **5**

3 $\xrightarrow[NaNH_2]{(C_6H_5)_3P^+\text{-}CH_3Br^-}$  **6**

$\xrightarrow[2)\ HCl/H_2O]{1)\ KOH/MeOH}$  **7**

4 $\xrightarrow[BF_3\text{-etherate}/CH_2Cl_2]{HS\text{-}(CH)_n\text{-}SH}$  **8**

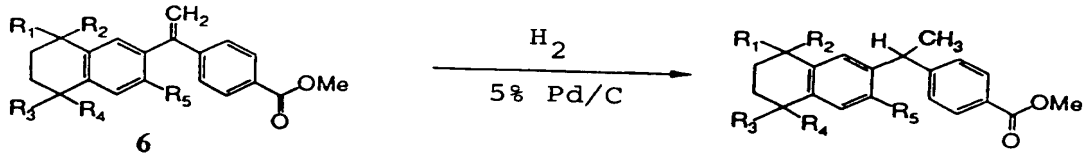
n=1 or 2

Treatment of ketone **4** with $NaBH_4$ afforded alcohol **5**.

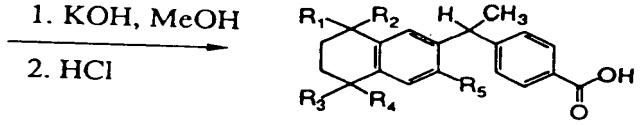
Treatment of the methyl ester **3** with methyltriphosphonium bromide-sodium amide in THF afforded the methano compound **6**.

The carboxylic acid **7** was formed by adding KOH to methano compound **6** in MeOH, followed by acidification.

The thioketals **8** were formed by treatment of **4** with 1,2-ethane dithiol or 1,3-propane dithiol and BF_3-etherate in CH_2Cl_2 at room temperature.



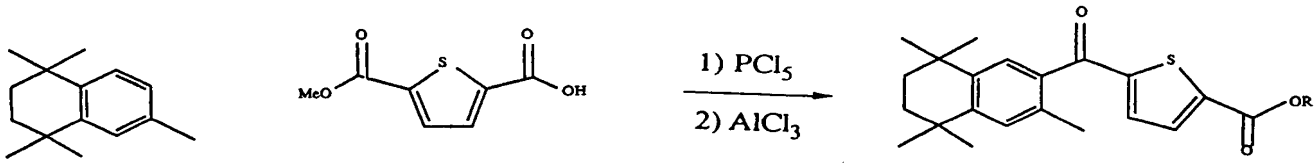
6 **9**



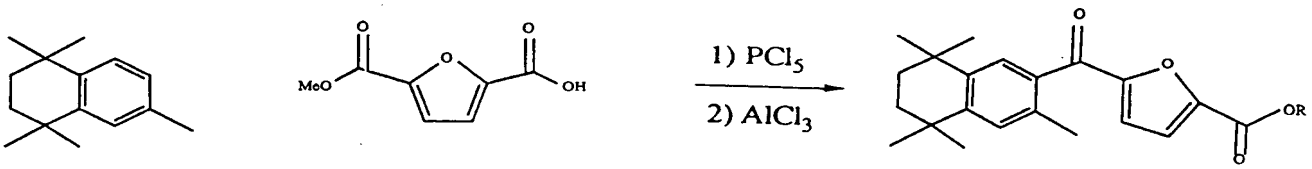
10

Treatment of the methyl ester **6** with hydrogen gas and 5% paladium over carbon in ethyl acetate yields the hydrogenated compound **9**.

Treatment of compound **9** with aqueous KOH in refluxing MeOH, followed by acidification, yields the carboxylic acid compound **10**.



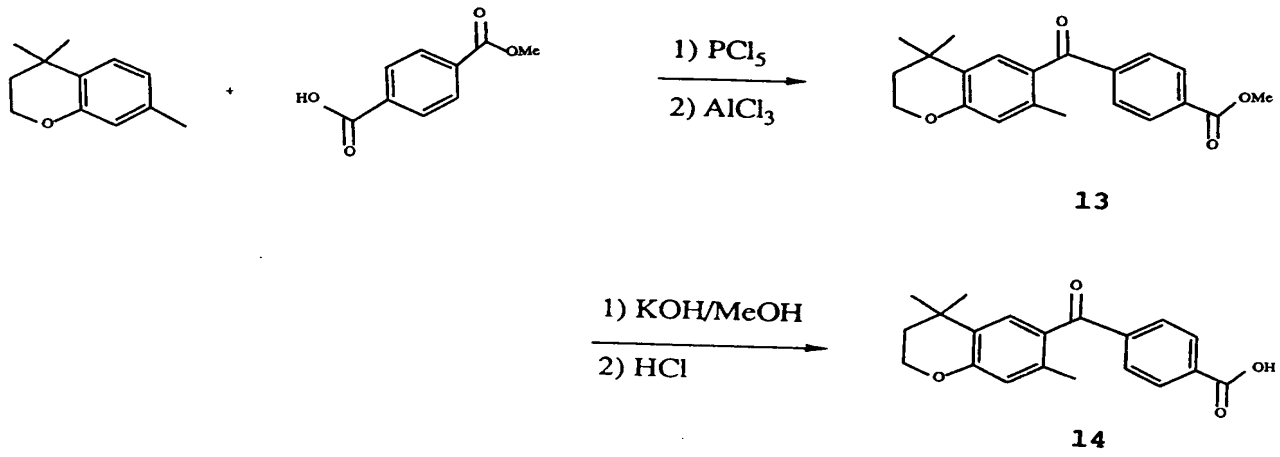
11



12

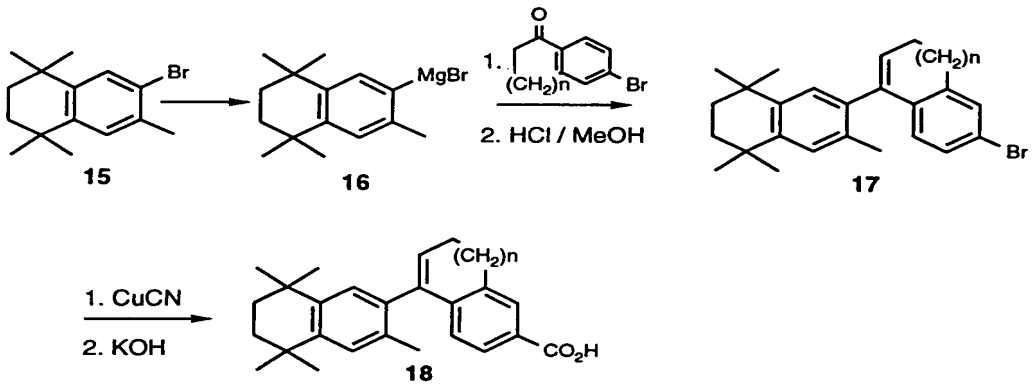
R = Me or H

Condensation of **1** with thiophene 2,5-mono methyl dicarboxylic acid or furanyl 2,5-mono methyl dicarboxylic acid was carried out by addition of PCl_5 in CH_2Cl_2 followed by addition of $AlCl_3$ at room temperature to give esters **11** and **12**, which were hydrolyzed with KOH followed by acidification to the corresponding acids.

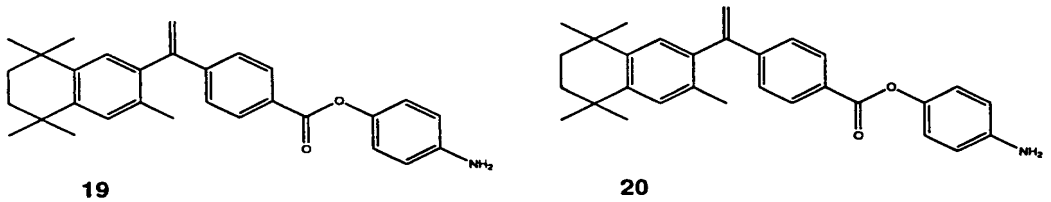


4,4 dimethylchroman and 4,4 dimethyl-7-alkylchroman compounds of type **13** and **14** as well as 4,4 dimethylthiochroman, 4,4 dimethyl 7-alkylthiochroman, 4,4 dimethyl-1,2,3,4-tetrahydroquinoline, and 4,4 dimethyl-7-alkyl-1,2,3,4-tetrahydroquinoline analogs were synthesized by similar methods as compound **3**, i.e., Friedal-Crafts conditions combining the appropriate dimethylchroman, dimethylthiochroman or dimethyltetrahydroquinoline with mono-methyl terephthalate acid chloride in the presence of $AlCl_3$ or $SnCl_4$ followed by base hydrolysis and acidification to give the carboxylic acid. For the synthesis of the tetrahydroquinoline analogs, it was necessary to acylate the amine before Friedel-Crafts coupling with mono-methyl

terephthalate acid chloride. For the synthesis of the appropriate dimethylchromans, dimethylthiochromans and tetrahydroquinolines, see U.S. Patent Nos. 5,053,523 and 5,023,341 and European Patent Publication No. 0284288.



Compounds of the type **18** were synthesized by nucleophillic addition of the Grignard reagent **16** to bromo tetralone, bromo indane, or other bicyclic ketone derivitive. Treatment of the resulting alcohol with methaolic HCl gave the intermediate **17**. Displacement of the bromine with CuCN in quinoline gave the nitrile which was then hydrolyzed to the acid **18** in refluxing KOH. Bromine compound **15** was synthesized from 2,5-dichloro-2,5, dimethyl hexane and 2-bromo toluene with a catalytic amount of $AlCl_3$.



19 **20**

Treatment of compounds 3-methyl-TTNCB and 3-methyl-TTNEB with DCC, p-amino phenol, and DMAP resulted in the amino-esters **19** (3-methyl-TTNCHBP) and **20** (3-methyl-TTNEHBP).

Illustrative examples for the preparation of some of the compounds according to this invention are as follows:

Example 1

Preparation of compound **3** where R_1, R_2, R_3, R_4 and R_5 are methyl, R' and R" are oxo, and X=COOMe:

To 7 gm (34.7 mmol) of 3-methyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl naphthalene and 6 gm (33.3 mmol) of mono-methyl teraphthalate in 200 mL of CH_2Cl_2 was added 8 g (38.8 mmol) of PCl_5. The reaction boiled vigorously and turned clear within 10 min. After stirring for an additional 1 h, 6 g (43.5 mmol) of $AlCl_3$ was added in 1 g portions over 15 min. and the reaction was allowed to stir overnight. The mixture was poured into 300 mL of 20% aqueous HCl and extracted with 5% EtOAc-hexanes, dried (MgSO4), concentrated, and crystallized from MeOH to give ca. 6 gm (16.5 mmol) of methyl ester **3**.

^{1}HNMR (CD_3OCD_3) δ 1.20 (s, 2(CH_3)), 1.35 (s, 2(CH_3)), 1.75 (s, 2(CH_2)), 2.31 (s, CH_3), 3.93 (s, $COOCH_3$), 7.21 (s, Na-CH), 7.23 (s, Na-CH), 7.85 (d, J=8 Hz, Ar-2(CH)), 8.18 (d, J=8 Hz, Ar-2(CH)).

Example 2

Preparation of compound **4** where R_1, R_2, R_3, R_4 and R_5 are methyl, R' and R" are oxo, and X = COOH:

To 6 gm (16.5 mmol) of methyl ester **3** suspended in 100 mL of MeOH was added 50 mL of 5N aqueous KOH. The mixture was heated under reflux for 1 h, cooled, acidified (20% aqueous HCl) and the organics extracted with EtOAc. After drying (MgSO4), the product was concentrated and precipitated from 1:4 EtOAc-hexanes to give ca. 5 g (14.3 mmol) of acid **4**.

^{1}HNMR (CD_3OCD_3) δ 1.20 (s, 2(CH_3)), 1.35 (s, 2(CH_3)), 1.75 (s, 2(CH_2)), 2.31 (s, CH_3), 7.21 (s, Na-CH), 7.23 (s, Na-CH), 7.91 (d, J=8 Hz, Ar-2(CH)), 8.21 (d, J=8 Hz, Ar-2(CH)).

Example 3

Preparation of compound **5** where R_1, R_2, R_3, R_4 and R_5 are methyl, R' = H and R" = OH, and X = COOH:

To a 1:1 THF-MeOH solution containing 1 g (2.86 mmol) of ketone **4** was added 100 mg of $NaBH_4$. The mixture was heated to 50°C for 10 min., cooled, acidified (20% aqueous HCl), and the organics extracted (EtOAc). After drying ($MgSO_4$), the product was concentrated and precipitated from 1:3 EtOAc-hexanes to give 550 mg (1.56 mmol) of the alcohol **5**.

^{1}HNMR (CD_3OCD_3) δ 1.20 (s, CH_3)), 1.22 (s,(CH_3)), 1.22 (s, 2(CH_3)), 1.65 (s, 2(CH_2)), 2.21 (s, CH_3), 6.00 (s, -CHOH-), 7.09 (s, Na-CH), 7.41 (s, Na-CH), 7.53 (d, J=8 Hz, Ar-2(CH)), 8.01 (d, J=8 Hz, Ar-2(CH)).

Example 4

Preparation of compound **6** where R_1, R_2, R_3, R_4 and R_5 are methyl, R' and R" are methano, and X = COOMe:

To 1 gm of methyl ester **3** (2.7 mmol) in 25 mL of dry THF was added 1.2 g (3.08 mmol) of methyltriphosphonium bromide-sodium amide. The solution was stirred at RT for 3 h or until complete by TLC (20% EtOAc-hexanes). Water was added and the organics were extracted with EtOAc, dried ($MgSO_4$), concentrated and purified by SiO_2 chromatography (5% EtOAc-hexanes) followed by crystallization from MeOH to give 700 mg (1.93 mmol) of methano compound **6**.

^{1}HNMR (CD_3OCD_3) δ 1.22 (s, 2(CH_3)), 1.30 (s, 2(CH_3)), 1.72 (s, 2(CH_2)), 1.95 (s, CH_3), 3.85 (s, $COOCH_3$), 5.29 (s, CH_2), 5.92 (s, CH_2), 7.19 (s, Na-CH), 7.20 (s, Na-CH), 7.39 (d, J=8 Hz, Ar-2(CH)), 7.96 (d, J=8 Hz, Ar-2(CH)).

Example 5

Preparation of compound **7** where R_1, R_2, R_3, R_4 and R_5 are methyl, R' and R" are methano, and X = COOH:

To 500 mg of methano compound **6** (1.38 mmol) in 20 mL of MeOH was added 5 mL of 5 N aqueous KOH and the suspension was refluxed for 1 h. After acidification (20% aqueous HCl) the organics were extracted (EtOAc), dried ($MgSO_4$), concentrated, and the solids recrystallized from EtOAc-hexanes 1:5 to give 350 mg (1.0 mmol) of the carboxylic acid **7**.

^{1}HNMR (CD_3OCD_3), δ 1.22 (s, 2(CH_3)), 1.30 (s, 2(CH_3)), 1.72 (s, 2(CH_2)), 1.95 (s, CH_3), 5.22 (s, CH_2), 5.89 (2, CH_2), 7.19 (s, Na-CH), 7.20 (s, Na-CH), 7.39 (d, J=8 Hz, Ar-2(CH)), 7.96 (d, J = 8 Hz, Ar-2(CH)).

Example 6

Preparation of compound **8** where R_1, R_2, R_3, R_4 and R_5 are methyl, R' and R" are $S(CH_2)_3S$, and X = COOH:

To 1 g (2.86 mmol) of ketone **4** in 20 mL of CH_2Cl_2 was added 500 mg (4.39 mmol) of 1,3-propane dithiol and excess BF_3-etherate. The reaction was stirred overnight, quenched with H_2O and the organics extracted with ether. The ether extract was dried ($MgSO_4$), concentrated, and the organics crystallized from warm hexane to give 850 mg (2.33 mmol) of the thioketal **8**.

Evaluation of Retinoid Receptor Subtype Selectivity

Representative synthetic retinoid compounds of the current invention were analyzed and found to exhibit subtype selectivity for retinoid receptors, and to be capable of modulating processes selectively mediated by retinoid X receptors, as discussed more fully below.

As employed herein, the phrase "processes selectively mediated by retinoid X receptors" refers to biological, physiological, endocrinological, and other bodily processes which are mediated by receptors or receptor combinations which are responsive to retinoid X receptor selective processes, e.g., compounds which selectively activate one and/or multiple members of the RXR subfamily. Modulation of such processes can be accomplished in vitro or in vivo. In vivo modulation can be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

The receptors which are responsive to retinoid X receptor selective ligands include: retinoid X receptor-alpha, retinoid X receptor-beta, retinoid X receptor-gamma, and splicing variants encoded by the genes for such receptors, as well as various combinations thereof (i.e., homodimers, homotrimers, heterodimers, heterotrimers, and the like). Also included are combinations of retinoid X receptors with other members of the steroid/thyroid superfamily of receptors with which the retinoid X receptors may interact by forming heterodimers, heterotrimers, and the higher heteromultimers. For example, the retinoic acid receptor-alpha, -beta, or -gamma isoforms may form a heterodimer with any of the retinoid X receptor isoforms, (i.e., alpha, beta, or gamma, including any combination of the different receptor isoforms), and the various retinoid X receptors may form a heterodimer with thyroid receptor or may form a heterodimer with vitamin D receptor. Members of the retinoid X receptor subfamily may form a heterodimer with certain "orphan receptors" including PPAR (Issemann and Green, Nature, 347:645-49 (1990)); HNF4 (Sladek et al., Genes & Development 4:2353-65 (1990)); the COUP family of receptors (e.g., Miyajima et al., Nucleic Acids Research 16:11057-74 (1988), and

Wang et al., Nature, 340:163-66 (1989)); COUP-like receptors and COUP homologs, such as those described by Mlodzik et al. (Cell, 60:211-24 (1990)) and Ladias et al. (Science, 251:5561-65 (1991)); the ultraspiracle receptor (e.g., Oro et al., Nature, 347:298-301 (1990)); and the like.

As employed herein, the phrase "members of the steroid/thyroid superfamily of receptors" (also known as "nuclear receptors" or "intracellular receptors") refers to hormone binding proteins that operate as ligand-dependent transcription factors. Furthermore, this classification includes identified members of the steroid/thyroid superfamily of receptors for which specific ligands have not yet been identified (referred to hereinafter as "orphan receptors"). All members of the intracellular receptor superfamily have the intrinsic ability to bind to specific DNA sequences. Following binding, the transcriptional activity of a target gene (i.e., a gene associated with the specific DNA sequence) is modulated as a function of the ligand bound to the receptor. Also, see copending U.S. Serial No. 809,980, whose entire disclosure is incorporated herein by reference.

The modulation of gene expression by the ligand retinoic acid and its receptors can be examined in a reconstituted system in cell culture. Such a system was used to evaluate the synthetic retinoid compounds of this invention for their interaction with the retinoid receptor subtypes RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ.

The system for reconstituting ligand-dependent transcriptional control, which was developed by Evans et al., Science, 240:889-95 (1988), has been termed a "co-transfection" or "cis-trans" assay. This assay is described in further detail in U.S. Patent Nos. 4,981,784 and 5,071,773, which are incorporated herein by reference. Also see Heyman et al., Cell, 68:397-406

(1992). The co-transfection assay provides a mechanism to evaluate the ability of a compound to modulate the transcription response initiated by an intracellular receptor. The co-transfection assay is a functional, rapid assay that monitors hormone or ligand activity and is a good predictor of an in vivo system.

Briefly, the co-transfection assay involves the introduction of two plasmids by transient transfection into a retinoid receptor-negative mammalian cell background. The first plasmid contains a retinoid receptor cDNA and directs constitutive expression of the encoded receptor. The second plasmid contains a cDNA that encodes for a readily quantifiable protein, e.g., firefly luciferase or chloramphenicol acetyl transferase (CAT), under control of a promoter containing a retinoid acid response element, which confers retinoid dependence on the transcription of the reporter. In this co-transfection assay, all retinoid receptors respond to all-trans-retinoic acid in a similar fashion. This assay can be used to accurately measure efficacy and potency of retinoic acid and synthetic retinoids as ligands that interact with the individual retinoid receptor subtypes.

Accordingly, synthetic retinoid compounds of the current invention were evaluated for their interaction with retinoid receptor subtypes using the co-transfection assay in which CV-1 cells were co-transfected with one of the retinoid receptor subtypes, a reporter construct, and an internal control to allow normalization of the response for transfection efficiency. The following example is illustrative.

Example 7

Retinoids: All-trans-retinoic acid (RA) and 13-cis-retinoic acid (13-cis-RA) were obtained from Sigma. 9-cis-retinoic acid (9-cis-RA) was synthesized as described in Heyman et al., Cell,

68:397-406 (1992). Retinoid purity was established as greater than 99% by reverse phase high-performance liquid chromatography. Retinoids were dissolved in dimethylsulfoxide for use in the transcriptional activation assays.

Plasmids: The receptor expression vectors used in the co-transfection assay have been described previously (pRShRAR-α: Giguere et al. (1987); pRShRAR-β and pRShRAR-γ: Ishikawa et al. (1990); pRShRXR-α: Mangelsdorf et al., (1990); pRSmRXR-β and pRSmRXR-γ: Mangelsdorf et al., Genes & Devel., 6:329-44 (1992)). A basal reporter plasmid Δ-MTV-LUC (Hollenberg and Evans, Cell, 55:899-906 (1988)) containing two copies of the TRE-palindromic response element 5'-TCAGGTCATGACCTGA-3' (Umesono et al., Nature, 336:262-65 (1988)) was used in transfections for the RARs, and CRBPIIFKLUC, which contains an RXRE (retinoid X receptor response element (Mangelsdorf et al., Cell, 66:555-61 (1991)), was used in transfections for the RXRs.

Co-transfection Assay In CV-1 Cells: A monkey kidney cell line, CV-1, was used in the cis-trans assay. Cells were transfected with two plasmids. The trans-vector allowed efficient production of the retinoid receptor in these cells, which do not normally express this receptor protein. The cis-vector contains an easily assayable gene product, in this case the firefly luciferase, coupled to a retinoid-responsive promoter, i.e., an RARE or RXRE. Addition of retinoic acid or an appropriate synthetic retinoid results in the formation of a retinoid-RAR or -RXR complex that activates the expression of luciferase gene, causing light to be emitted from cell extracts. The level of luciferase activity is directly proportional to the effectiveness of the retinoid-receptor complex in activating gene expression. This sensitive and

reproducible co-transfection approach permits the identification of retinoids that interact with the different receptor isoforms.

Cells were cultured in DMEM supplemented with 10% charcoal resin-stripped fetal bovine serum, and experiments were conducted in 96-well plates. The plasmids were transiently transfected by the calcium phosphate method (Umesono and Evans, Cell, 57:1139-46 (1989) and Berger et al., J. Steroid Biochem. Molec. Biol., 41:733-38 (1992)) by using 10 ng of a pRS (Rous sarcoma virus promoter) receptor-expression plasmid vector, 50 ng of the reporter luciferase (LUC) plasmid, 50 ng of pRSβ-GAL(β-galactosidase) as an internal control, and 90 ng of carrier plasmid, pGEM. Cells were transfected for 6 h and then washed to remove the precipitate. The cells were then incubated for 36 h with or without retinoid. After the transfection, all subsequent steps were performed on a Beckman Biomek Automated Workstation. Cell extracts were prepared, then assayed for luciferase and β-galactosidase activities, as described by Berger et al. (1992). All determinations were performed in triplicate in two independent experiments and were normalized for transfection efficiency by using β-galactosidase as the internal control. Retinoid activity was normalized relative to that of all-trans-retinoic acid and is expressed as potency (EC50), which is the concentration of retinoid required to produce 50% of the maximal observed response, and efficacy (%), which is the maximal response observed relative to that of all-trans-retinoic acid at 10^{-5}M. The data obtained is the average of at least four independent experiments. Efficacy values less than 5% are not statistically different than the 0% background. Compounds with an efficacy of less than 20% at concentrations of 10^{-5} M are considered to be inactive. At higher concentrations of compound, such as 10^{-4} M, these compounds are

generally toxic to cells and thus the maximal efficacy at 10^{-5} M is reported in the tables and figures contained herein.

The synthetic retinoid compound 3-methyl TTNCB, as described above, was evaluated for its ability to regulate gene expression mediated by retinoid receptors. As shown in Figure 1, this compound is capable of activating members of the RXR subfamily, i.e., RXRα, RXRβ, and RXRγ, but clearly has no significant activity for members of the RAR subfamily, i.e., RARα, RARβ, and RARγ. Assays using all-trans-retinoic acid (Figure 2) and 9-cis-retinoic acid (Figure 3) were run for reference, and demonstrate that these retinoic acid isomers activate members of both the RAR and RXR subfamilies.

Potency and efficacy were calculated for the 3-methyl-TTNCB compound, as summarized in the following table. For reference, the data for 9-cis-retinoic acid are also included.

TABLE 1

	Potency (nM)	Efficacy
3-Methyl-TTNCB		
RXRα	330	130%
RXRβ	200	52%
RXRγ	260	82%
RARα	>10,000	<2%
RARβ	>10,000	<4%
RARγ	>10,000	<4%
9-cis-retinoic acid		
RXRα	150	140%
RXRβ	100	140%
RXRγ	110	140%
RARα	160	100%
RARβ	5	82%
RARγ	47	120%

As shown by the data in Table 1, 3-methyl-TTNCB readily and at low concentrations activates RXRs. Further, 3-methyl-TTNCB is more potent an activator of RXRs than RARs, and preferentially activates RXRs in comparison to RARs, in that much higher concentrations of the compound are required to activate the RARs. In contrast, 9-cis-retinoic acid does not preferentially activate the RXRs, as also shown in Table 1. Rather, 9-cis-retinoic acid activates the RARβ and RARγ isoforms at lower concentrations and more readily than the RXRβ and RXRγ isoforms, and has substantially the same, within the accuracy of the measurement, activity for the RARα isoform in comparison to the RXRα isoform.

An extract reported to contain 9-cis-retinoic acid has previously been reported as at least 10-fold more potent in inducing RXRα than RARα (Heyman et al., Cell, 68:397,399 (January 24, 1992)). Presently available data indicate that 9-cis-retinoic acid does not preferentially activate RXRs in comparison to RARs, as shown and discussed above. The compounds of this invention preferentially activate RXRs in comparison to RARs, and are preferably at least several times more potent as activators of RXRs than RARs.

Potency and efficacy have also been calculated for the 3-methyl-TTNEB, 3-bromo-TTNEB, 3-methyl-TTNCHBP, 3-methyl-TTNEHBP, and TPNEP compounds, as summarized below in Table 2.

TABLE 2

	Potency (nM)	Efficacy
3-Methyl-TTNEB		
RXRα	40	83%
RXRβ	21	102%
RXRγ	34	80%
RARα	>10,000	6%
RARβ	>10,000	17%
RARγ	>10,000	19%
3-Bromo-TTNEB		
RXRα	64	88%
RXRβ	54	49%
RXRγ	52	71%
RARα	>10,000	3%
RARβ	>10,000	18%
RARγ	>10,000	15%
3-Methyl-TTNCHBP		
RXRα	1100	113%
RXRβ	1100	155%
RXRγ	300	128%
RARα	>10,000	<2%
RARβ	>10,000	7%
RARγ	>10,000	17%
3-Methyl-TTNEHBP		
RXRα	140	125%
RXRβ	71	121%
RXRγ	48	163%
RARα	>10,000	<2%
RARβ	1,900	25%
RARγ	>10,000	10%
TPNEP		
RXRα	5	75%
RXRβ	5	138%
RXRγ	6	100%
RARα	>10,000	<2%
RARβ	>10,000	<2%
RARγ	1,500	24%

As shown by the data in Table 2, 3-methyl-TTNEB, 3-bromo-TTNEB, 3-methyl-TTNCHBP, 3-methyl-TTNEHBP, and TPNEP each readily and preferentially activate the RXRs, and are more potent as activators of RXRs than of RARs. The diminished activity of these compounds for the RARs in comparison to the RXRs is also shown for some of these compounds in Figures 4-7.

It can be expected that synthetic retinoid ligands, such as those exemplified in Tables 1 and 2 which preferentially affect some but not all of the retinoic acid receptor isoforms, can, in pharmacological preparations, provide pharmaceuticals with higher therapeutic indices and a better side effect profile than currently used retinoids. For example, the compounds of the present invention have been observed to be less irritating than standard retinoids.

The retinoid compounds of this invention are useful for the treatment of certain dermatological conditions such as keratinization disorders, i.e., differentiation/ proliferation. A standard assay to determine the activity of these compounds is the measurement of the enzymatic activity for transglutaminase; this is a measure of the antiproliferative action of retinoids. Retinoids have been shown to inhibit the pathway of differentiation, which is indicated by a decrease in several biochemical markers that are associated with the expression of squamous cell phenotype, such as transglutaminase. (Yuspa et al., Cancer Research, 43:5707-12 (1983)). As can be seen from Figure 8, the 3-methyl-TTNCB compound is capable of inhibiting transglutaminase activity and inhibits 50% of the enzyme activity at 1×10^{-7} M.

The compounds of this invention also exhibit good comedolytic activity in the test on Rhino mice described by Kligman et al. (J. of Inves. Derm., 73:354-58 (1979)) and Mezick et al. (J.

of Inves. Derm., 83:110-13 (1984)). The test on Rhino mice has been a model for screening comedolytic agents. The activity of the 3-methyl-TTNCB retinoid compound, as well as 9-cis and all-trans retinoic acid is shown in Figure 9. A 0.1% solution of 3-methyl-TTNCB is capable of inhibiting the utriculi diameter by approximately 50%. It has also been observed that 3-methyl-TTNCB is less irritating to the Rhino mice than 9-cis- or all-trans-retinoic acid.

The synthetic retinoids of the current invention have also been tested using radioligand displacement assays. RAR and RXR isoforms overexpressed in E. coli or baculovirus are capable of binding radiolabeled 9-cis-retinoic acid with binding parameters which are essentially similar to those receptors overexpressed in mammalian cells. By testing the abilities of various synthetic retinoids to compete with the radiolabeled retinoic acid for binding to various receptor isoforms, the relative dissociation constant for the receptor itself can be determined. This is an important supplementary analysis to the co-transfection assay since it can detect important discrepancies that may arise due to the various determinants of retinoid activity in the co-transfection assay. These determinants may include (1) activating or inactivating metabolic alterations in the test compounds, (2) binding to serum proteins which alter the free concentration of the test compound, (3) differences in cell permeation among test compounds, (4) intrinsic differences in the affinity of the test compounds for the receptor proteins, i.e., in K_d, and (5) conformational changes produced in the receptor after binding of the test compound, reflected in the effects on reporter gene expression.

The 3-methyl-TTNCB compound is capable of displacing ^3H-9-cis-retinoic acid bound to the RXRs, but is not capable of

displacing radiolabeled ligand that is bound to the RARs. This indicates that the 3-methyl-TTNCB compound preferentially binds RXRs in comparison to RARs, a property which would be expected of a ligand selective for the RXRs.

It has been recognized that the co-transfection assay provides a functional assessment of the ligand being tested as either an agonist or antagonist of the specific genetic process sought to be affected. Ligands which do not significantly react with other intracellular receptors, as determined by the co-transfection assay, can be expected to result in fewer pharmacological side effects. Because the co-transfection assay is run in living cells, the evaluation of a ligand provides an early indicator of the potential toxicity of the candidate at concentrations where a therapeutic benefit would be expected.

Processes capable of being modulated by retinoid receptors, in accordance with the present invention, include in vitro cellular differentiation, the regulation of morphogenetic processes including limb morphogenesis, regulation of cellular retinol binding protein (CRBP), and the like. As readily recognized by those of skill in the art, the availability of ligands for the retinoid X receptor makes it possible, for the first time, to elucidate the processes controlled by members of the retinoid X receptor subfamily. In addition, it allows development of assays for the identification of antagonists for these receptors.

The processes capable of being modulated by retinoid receptors, in accordance with the present invention, further include the in vivo modulation of lipid metabolism; in vivo modulation of skin related processes (e.g., acne, psoriasis, aging, wrinkling, and the like); in vivo modulation of malignant cell

development, such as occurs, for example, in acute promyelocytic leukemia, mammary cancer, prostate cancer, lung cancer, cancers of the aerodigestive pathway, skin cancer, bladder cancer, and sarcomas; in vivo modulation of premalignant lesions, such as occurs with oral leukoplakia and the like; in vivo modulation of auto-immune diseases such as rheumatoic arthritis; in vivo modulation of fatty acid metabolism; and the like. Such applications can be expected to allow the modulation of various biological processes with reduced occurrence of undesirable side effects such as teratogenic effects, skin irritation, mucosal dryness, lipid disturbances, and the like. In vivo applications can be employed with a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

For example, regarding the in vivo modulation of lipid metabolism referred to above, apolipoprotein AI is a major protein component of plasma high density lipoprotein (HDL) cholesterol. Since the circulating level of HDL in humans has been shown to be inversely correlated to the risk of coronary vascular diseases, it can be expected that regulating synthesis of apolipoprotein AI can be utilized in the treatment of coronary vascular disease. It has been established that regulation of transcription of apolipoprotein AI is controlled by members of the intracellular receptor superfamily, and further that the apolipoprotein AI gene transcription start site A is a highly selective retinoid responsive element (RXRE) that responds preferentially to RXRα. Rottman et al., Mol. Cell. Biol., 11:3814-20 (1991). Therefore, ligands which selectively activate members of the RXR family of retinoic acid receptors are regulators of apolipoprotein AI transcription. We have demonstrated in in vivo studies that ligands having selective activity for RXRs can be used to

significantly raise plasma HDL levels, as demonstrated in the following example.

Example 8

Male Sprague-Dawley rats (160-200 gram) were obtained from Harland. Animals were fed standard laboratory diets (Harlan/Teklad) and kept in an environmentally controlled animal house with a light period lasting from 6 a.m. to 6 p.m. Animals were treated with drugs prepared as suspensions in olive oil.

To verify that RXR activation can modulate HDL cholesterol, an initial study was carried out that included dosing rats for 4 days with an RAR-selective compound, all-trans retinoic acid, the non-selective RAR/RXR agonist, 9-cis-retinoic acid, and either of two RXR-selective agents, 3-methyl-TTNCB or 3-methyl-TTNEB. Each drug was administered at a dose of 100 mg/kg, i.p. Positive control groups received olive oil as a vehicle. Twenty-four hours after the last treatment, rats were sacrificed by CO_2 inhalation, blood was collected from the inferior vena cava into a tube containing 0.1 ml of 0.15% EDTA and centrifuged at 1500 x g for 20 min. at 4°C. Plasma was separated and stored at 4°C for evaluation of plasma total cholesterol and high density lipoprotein cholesterol (HDL-cholesterol).

Plasma total cholesterol was measured enzymatically utilizing Boeringer Mannheim Diagnostics High Performance Cholesterol Methods with an ABBOTT VP Bichromatic Analyzer. HDL was measured after preparation of the HDL-containing fraction by heparin-manganese precipitation of plasma. HDL-cholesterol in this fraction was estimated as mentioned earlier. All HDL separations were checked for contamination by either lipoproteins with agarose gel electrophoresis.

The results of this study are shown in Figure 10. As shown, rats receiving the RXR-selective compounds exhibited substantial

and statistically significant increases in HDL levels, particularly when receiving 3-methyl-TTNEB. Because the RXR-selective ligand 3-methyl-TTNEB was the most efficacious, additional 4 day experiments were conducted with this agent at doses of 0.3, 1, 3, 6, 10, 30, 100, or 300 mg/kg i.p. in 0.5 ml olive oil or 1, 3, 10, 30, 100, 300 mg/kg p.o. in 0.5 ml olive oil for 4 days. An additional 30 day p.o. study was conducted with 10, 30, or 100 mg/kg 3-methyl-TTNEB to determine whether tolerance would develop to its pharmacological actions. For the rats receiving 3-methyl-TTNEB in various doses for four days, it was also observed that most of the HDL elevation was obtainable with relatively low doses (less than 5 mg/kg) of 3-methyl-TTNEB. The 30-day study with 3-methyl-TTNEB did not indicate development of tolerance to its pharmacological action.

Additional in vitro studies were also performed utilizing the co-transfection assay previously described within this application to demonstrate the effect of RXR-selective ligands on regulation of transcription of apolipoprotein-AI, as described in the following example.

Example 9:

This work focused on studying the transcriptional properties of the retinoid receptors RAR and RXR on a reporter molecule (e.g., luciferase) under control of a basal promoter containing the RXR response element from the apolipoprotein AI gene ("A" site). Plasmid constructs coding for the various receptors were transfected into a human hepatocyte cell line (HepG-2) along with the reporter plasmid. Reporter plasmids contained multimers of the apolipoprotein-AI "A" site (-214 to -192 relative to transcription start site) shown to bind RXR. Widom et al., Mol. Cell. Biol. 12:3380-89 (1992); Ladias & Karathanasis,

Science 251:561-65. After transfection, treatment, harvest, and assay, the data obtained was normalized to transfected beta-galactosidase activity so as to control for transfection efficiency. The results demonstrated activation in the system with the RXR-specific ligands 3-methyl-TTNCB and 3-methyl-TTNEB, demonstrating that the RXR specific ligands could regulate the transcriptional properties via the "A" site from the apolipoprotein AI gene. These compounds had no effect when RAR was used in the transfection, demonstrating receptor specificity. The transcriptional regulation by RXR was dependent on the presence of the hormone response element.

As will be discernible to those skilled in the art, the compounds disclosed can be readily utilized in pharmacological applications where selective retinoid X receptor activity is desired, and where it is desired to minimize cross reactivities with other related intracellular receptors. In vivo applications of the invention include administration of the disclosed compounds to mammalian subjects, and in particular to humans.

The compounds of the present invention are small molecules which are relatively fat soluble or lipophilic and enter the cell by passive diffusion across the plasma membrane. Consequently, these ligands are well suited for administration orally and by injection, as well as topically. Upon administration, these ligands can selectively activate retinoid X receptors, and thereby selectively modulate processes mediated by these receptors.

The pharmaceutical compositions of this invention are prepared in conventional dosage unit forms by incorporating an active compound of the invention, or a mixture of such compounds, with a nontoxic pharmaceutical carrier according to accepted

procedures in a nontoxic amount sufficient to produce the desired pharmacodynamic activity in a mammalian and in particular a human subject. Preferably, the composition contains the active ingredient in an active, but nontoxic, amount selected from about 5 mg to about 500 mg of active ingredient per dosage unit. This quantity depends on the specific biological activity desired and the condition of the patient.

The pharmaceutical carrier or vehicle employed may be, for example, a solid or liquid. A variety of pharmaceutical forms can be employed. Thus, when using a solid carrier, the preparation can be plain milled, micronized in oil, tableted, placed in a hard gelatin or enteric-coated capsule in micronized powder or pellet form, or in the form of a troche, lozenge, or suppository. When using a liquid carrier, the preparation can be in the form of a liquid, such as an ampule, or as an aqueous or nonaqueous liquid suspension. For topical administration, the active ingredient may be formulated using bland, moisturizing bases, such as ointments or creams. Examples of suitable ointment bases are petrolatum, petrolatum plus volatile silicones, lanolin, and water in oil emulsions such as Eucerin (Beiersdorf). Examples of suitable cream bases are Nivea Cream (Beiersdorf), cold cream (USP), Purpose Cream (Johnson & Johnson) hydrophilic ointment (USP), and Lubriderm (Warner-Lambert).

The following examples provide illustrative pharmacological composition formulations:

Example 10

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
3-methyl-TTNCB	140
Starch, dried	100
Magnesium stearate	10
Total	250 mg

The above ingredients are mixed and filled into hard gelatin capsules in 250 mg quantities.

Example 11

A tablet is prepared using the ingredients below:

	Quantity (mg/tablet)
3-methyl-TTNCB	140
Cellulose, microcrystalline	200
Silicon dioxide, fumed	10
Stearic acid	10
Total	360 mg

The components are blended and compressed to form tablets each weighing 360 mg.

Example 12

Tablets, each containing 60 mg of active ingredient, are made as follows:

	Quantity (mg/tablet)
3-methyl-TTNCB	60
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (PVP) (as 10% solution in water)	4
Sodium carboxymethyl starch (SCMS)	4.5
Magnesium stearate	0.5
Talc	1.0
Total	150 mg

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of PVP is mixed with the resultant powders, which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The SCMS, magnesium stearate, and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Example 13

Suppositories, each containing 225 mg of active ingredient, may be made as follows:

3-methyl-TTNCB	225 mg
Saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously

melted using the minimum heat necessary. The mixture is then poured into a suppository mold of normal 2g capacity and allowed to cool.

Example 14

An intravenous formulation may be prepared as follows:

3-methyl-TTNCB	100 mg
Isotonic saline	1,000 ml
Glycerol	100 ml

The compound is dissolved in the glycerol and then the solution is slowly diluted with isotonic saline. The solution of the above ingredients is then administered intravenously at a rate of 1 ml per minute to a patient.

The compounds of this invention also have utility when labeled as ligands for use in assays to determine the presence of RXRs. They are particularly useful due to their ability to selectively bond to members of the RXR subfamily and can therefore be used to determine the presence of RXR isoforms in the presence of other related receptors.

Due to the selective specificity of the compounds of this invention for retinoid X receptors, these compounds can also be used to purify samples of retinoid X receptors in vitro. Such purification can be carried out by mixing samples containing Retinoid X Receptors with one of more of the bicyclic derivative compounds disclosed so that the compound (ligand) binds to the receptor, and then separating out the bound ligand/receptor combination by separation techniques which are known to those of skill in the art. These techniques include column separation, filtration, centrifugation, tagging and physical separation, and antibody complexing, among others.

While the preferred embodiments have been described and illustrated, various substitutions and modifications may be made thereto without departing from the scope of the invention. Accordingly, it is to be understood that the present invention has been described by way of illustration and not limitation.